

Review

# Perinatal Epigenetic Determinants of Cognitive and Metabolic Disorders

Daniel S. Lupu<sup>1</sup>, Diana Tint<sup>2</sup>, Mihai D. Niculescu<sup>1,\*</sup>

<sup>1</sup>UNC Nutrition Research Institute, Department of Nutrition, University of North Carolina at Chapel Hill, Kannapolis, NC, U.S.A.

<sup>2</sup>School of Medicine, Transilvania University in Brasov, Brasov, Romania

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**ABSTRACT:** Multiple cues from the environment of our indirect and immediate ancestors, which often persist throughout the prenatal period and adulthood, are shaping our phenotypes through either direct, parent-to-child influences, or transgenerational inheritance. These effects are due to gene-environment interactions, which are intended to be a predictive tool and a mechanism of quick adaptation to the environment, as compared with genetic variations that are inherited over many generations. In certain circumstances the influences induced by the gene-environment interactions can have deleterious effects upon the health status, in the context of a radical change in the environment that does not fit with the predicted conditions, via epigenetic alterations. Conversely the best fit to the *expected* environment might have a delayed aging process and a longer life span. This review will touch upon the Developmental Origins of Health and Disease (DoHAD) concept, while discussing recent advances in the understanding of metabolic and cognitive disruptions, with a focus on epigenetic factors, their transgenerational effects, and the consequences they might have upon the onset of chronic disease and premature exitus.

**Key words:** Metabolism, cognition, epigenetic, transgenerational

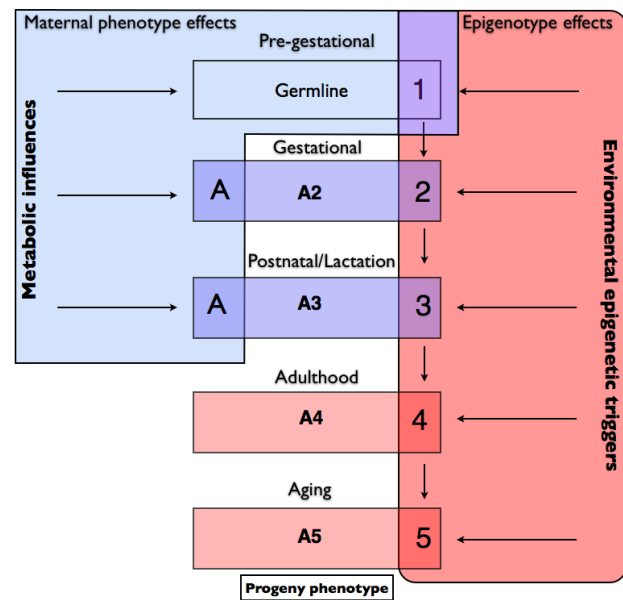
We cannot discuss aging, as a period with increased susceptibility to disease and death due to cellular senescence [1], without considering epigenetic factors that are involved in regulating gene expression [2]. This is a relatively new addition to the list of the investigated determinants that are incriminated in senescence, such as genetic background, telomerase activity, and immunologic collapse [3-5]. We should conceptually distinguish between a constant, somatic and cognitive decline (physiological aging), and an accelerated process of losing survival functions, associated with early chronic disease (pathological aging) [5, 6]. For instance, the severe cognitive impairment associated with Alzheimer's disease, as compared to mild cognitive impairment, can be equivalent to 22.7 and 15 years,

respectively, of cognitive performance decline in healthy aging individuals [6]. Epigenetic alterations, initiated during the life of our ancestors and during different life stages of our own existence, in tandem with maternal phenotypic effects, can contribute to this premature aging phenotype, or facilitate physiological senescence through their modulating potential (Fig. 1). Such factors include DNA methylation, micro RNA and polycomb group proteins, which modulate chromatin structure through histone modifications, determining an age-related phenotype (Fig. 2). The susceptibility of acquiring chronic diseases or, conversely, the potential to delay their onset and death, have a clear epigenetic component [7-9]. This epigenetic layer of gene regulation adds to the genetic, neuroendocrine,

\*Correspondence should be addressed to: Dr. Mihai D. Niculescu. UNC Nutrition Research Institute, 500 Laureate Way, Kannapolis, NC 28081, USA. Email: [Mihai\\_Niculescu@unc.edu](mailto:Mihai_Niculescu@unc.edu).

psychological, behavioral/social, and immunologic models that impact upon the parent generation, and contributes to the phenotype shaping in the next generation(s). The relationship between the environmental exposure of parents, and the inheritance of the acquired traits via epigenetic mechanisms by the next generation, was hypothesized to be an attempt for improving degree of fitness in the offspring, as related to a predicted environment [10]. However, a potential mismatch between the maternally-programmed behavior of the offspring, and the actual environment, will create a disadvantage that will contribute to the risk of acquiring a chronic disease later in life (DoHAD, Developmental origins of Health and Disease) [11]. This less fitted phenotype might be decisive in switching the balance from physiological to pathological aging. For example, the drive for food overconsumption instilled during times with scarce resources can be transmitted to a progeny exposed to an environment with either abundant resources or energy dense foods, with negative metabolic and cardiovascular consequences [12]. In addition, the moment of the exposure could play an important role, with different vulnerabilities in early versus late pregnancy, or during the postnatal period [13]. This type of adaptive learning can be sex dependent and potentially reversible [14, 15], which brings biologic plausibility to the hypothesis stating that later interventions could offset, in part, the early-induced alterations of the phenotype.

Literature reviews indicated that, among the factors that contribute to morbidity in an aging population, the most important are those related to cardiovascular diseases [16], which are also closely linked with metabolic syndrome, one of the main drivers of premature aging [17]. Other important contributors, with a high impact upon quality of life and healthcare expenses, are related to cognitive decline, especially dementia [18]. These conditions are potentially preventable, with the perhaps intriguing observation that the timeline for prophylaxis should be extended at least to the perinatal period (Fig. 3) [19]. In this review we will refer to a substantial number of recent studies looking at the impact of a disruptor present before or during pregnancy, and the demonstrated effects starting from the first generation and beyond. We will review studies that investigate how factors like nutrition, stress, and chemical agents have a transgenerational effect with lifelong consequences. Then we will discuss the impact of various maternal physiopathologic factors on shaping the perinatal setting and defining the adult context of disease vulnerability from the behavioral/cognitive and metabolic perspectives, according to the paradigm of adaptive epigenetic responses.



**Figure 1. Determinants of the progeny phenotype.** The phenotype of the progeny (A2 to A5) is determined throughout different developmental periods by a combination of maternal metabolic influences upon progeny's phenotype (A), and epigenetic alterations acquired under the influence of environmental triggers (assuming, for this example, specific direct triggers for each period). The epigenetic status (1 to 5) will evolve during life stages, with the maternal phenotype also shaping the epigenetic setting during the germline period. For each generation, the resulting phenotype (A2 to A5) will result from the combination of maternal metabolic influences and the epigenetic events triggered by environmental exposures.

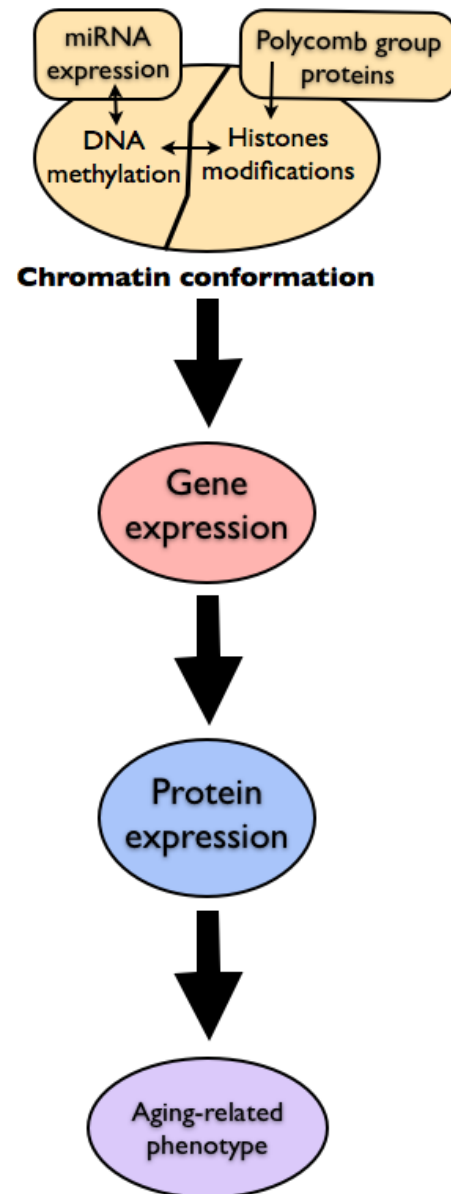
### Behavioral and cognitive consequences

Transgenerational inheritance can be detected over one or more generations. From an epigenetic perspective, a true transgenerational inheritance can be positively identified by studying the epigenetic impact of F0 epigenetic background upon the F3 generation (where the mother (F0), the embryo (F1) and the embryo germline (F2) might be subjected to the same environment during the F1 in-utero development). When discussing epigenetic transgenerational inheritance, it is important to note that, in order to demonstrate the effect, the original epigenetic triggers that initiated the epigenetic changes in F0 generation, should not be present in the generation hypothesized to inherit the epigenetic background. Thus, the first generation free of such direct influences is F3.

Nutrition, maternal stress, xenobiotics, and other factors that impact upon the perinatal environment are accepted determinants of later life patterns of psychiatric disease susceptibility, addiction, stress response, memory, and cognitive capacity (Table 1). Recent studies indicated that stressing the immature rat female

will increase anxiety and suppress social interaction in female offspring, but will improve habituation to fear in male pups [20]. Enrichment of both the maternal environment and, especially, progeny's environment, reduced anxiety and fear, and improved avoidance learning in the progeny group. Interestingly, a diet lacking in methyl donors during fetal development had the similar effect of increasing anxiety in the mature female rat offspring versus the control group, but with an additional beneficial response of higher learning ability in new environmental conditions [21]. This, however, was not explained by DNA methylation differences in four investigated hippocampal genes coding for glucocorticoid receptor, hydroxysteroid dehydrogenase 11 type 2, neuronatin, and reelin proteins. Similarly, chickens from parents exposed to certain stressors exhibited a higher coping ability when they were also subjected to stress through a sex specific reduced corticosterone response and changes in the expression of 44 genes, although parental care was absent [22]. In humans, maternal prenatal psychological stress and elevated cortisol levels in a cohort of 125 full term infants were predictive of a slower rate of development over the first year of life and lower mental development scores at 12 months if present early in gestation [23]. However, if the cortisol level was high in late gestation, the infants had better scores in tests assessing mental development [23]. Anxiety-like behavior transmitted over generations can be determined by multiple factors, such as xenobiotics and bacteria. Neonatal exposure to *Salmonella enteritidis* lipopolysaccharide (LPS) increased the susceptibility to increased anxiety through both maternal and paternal lines in the F2 generation of Wistar rats, with a higher corticosterone response to stress in females only [24]. Interestingly, this bacterial-modulated anxiety phenotype was reversed by cross fostering with unexposed dams. A potential marker for this type of behavior, as well as schizophrenia and autism, is reduced levels of glutamic acid decarboxylase 67 (GAD67) and reelin in the GHABA neurons of progeny hippocampi from the dams exposed during gestation [25]. Skinner et al showed that subjecting gestating rats to high doses of the fungicide vinclozolin modified the stress response of adolescent males over four generations without abatement, adding more weight to the theory that an initial exposure can have a true transgenerational effect, with the F3 generation being the first one not directly subjected to the environmental trigger [26]. Testing another endocrine disruptor, in concentrations similar to human exposure, another study showed that bisphenol A (BPA) addition in the diet of the F0 generation of C57BL/6J mice 7-10 days before mating correlated with higher expression of four estrogen or estrogen related receptors in whole brains of

the F1 generation, as confirmed by genome wide gene expression and qPCR [27]. Interestingly, the effects were different across generations, with the F1 group presenting decreased social interaction when compared with controls, but with the inverse effect on the F2 and F4 generations. The F3 generation was not tested for this study.



**Figure 2. Epigenetic mechanisms.** Inter-related epigenetic mechanisms modulate chromatin conformation changes that will eventually lead to an aging-related phenotype.

**Table 1. Transgenerational effects of various triggers on different animal models on behavior, metabolism, fertility and hormonal setup.**

| Trigger        |   | Response   |   | Ref. |
|----------------|---|--|---|------|
| Generation     | Type  | Generation/<br>Animal model                              | Type  |      |
| F <sub>0</sub> | Pre-reproductive stress<br>(27 -29 days dams)   | F <sub>1</sub><br>rats                                   | Increased generalized anxiety, improved avoidance learning, habituation to fear in males, partially reversed by postnatal enrichment  | [20] |
| F <sub>1</sub> | Intermittent social isolation in the first 3 weeks of life  | F <sub>2</sub><br>chickens                               | Higher growth, reduced corticosterone response with higher coping ability   | [22] |
| F <sub>0</sub> | Vinclozolin on gestating females<br>(on E8-14 of pregnancy)   | F <sub>3</sub><br>rats                                   | Weight gain, altered response to chronic restraint stress, increased anxiety  | [26] |
| F <sub>0</sub> | Diet with high methylating micronutrients<br>(35 days boars)  | F <sub>2</sub><br>pigs                                   | Lower backfat percentage with concurring gene expression in lipid metabolism and metabolic pathways in liver and muscle   | [52] |
| F <sub>0</sub> | Diet with bisphenol A<br>(7-10 days before pairing with a male)   | F <sub>1</sub> , F <sub>2</sub> , F <sub>4</sub><br>mice | Increased social interaction and decreased non-social behavior in F <sub>2</sub> and F <sub>4</sub> with an inverse effect on F <sub>1</sub>  | [27] |
| F <sub>0</sub> | High fat diet<br>(5 weeks old)  | F <sub>1</sub> , F <sub>2</sub><br>mice                  | Reduced sperm motility with increased levels of ROS; oocytes with increased oxidative stress and altered mitochondrial function   | [62] |
| F <sub>0</sub> | Diet rich in methionine pathway metabolites<br>(3 weeks before mating)  | F <sub>1</sub><br>mice                                   | Hypermethylated T cells with suppression of chemotaxis, lower TNF $\alpha$ , IL-2, IL-4   | [65] |
| F <sub>0</sub> | Maternal and/or paternal high fat diet<br>(female diet starting at week 8)  | F <sub>1</sub> , F <sub>2</sub> , F <sub>3</sub><br>mice | Increased body length and weight, impaired insulin sensitivity, decreased methylation in the GHSR promoter in F <sub>1</sub> ,F <sub>2</sub> with maintained phenotypic changes in F <sub>3</sub> (paternal lineage only) | [53] |
| F <sub>0</sub> | Daily IP injection with combinations of BPA, DEHP, DBP, DEET, permethrin, dioxin, and jet fuel<br>(embryonic days 8 – 14) | F <sub>1</sub> , F <sub>2</sub> , F <sub>3</sub><br>rats | Spermatogenic cell apoptosis, decrease of primordial follicle pool, early onset of puberty  | [63] |

**Abbreviations:** ROS (reactive oxygen species), TNF (tumor necrosis factor), BPA (bisphenol A), DEHP (bis (2-ethylhexyl) phthalate), DBP (dibutyl phthalate), DEET (N,N-Diethyl-meta-toluamide).

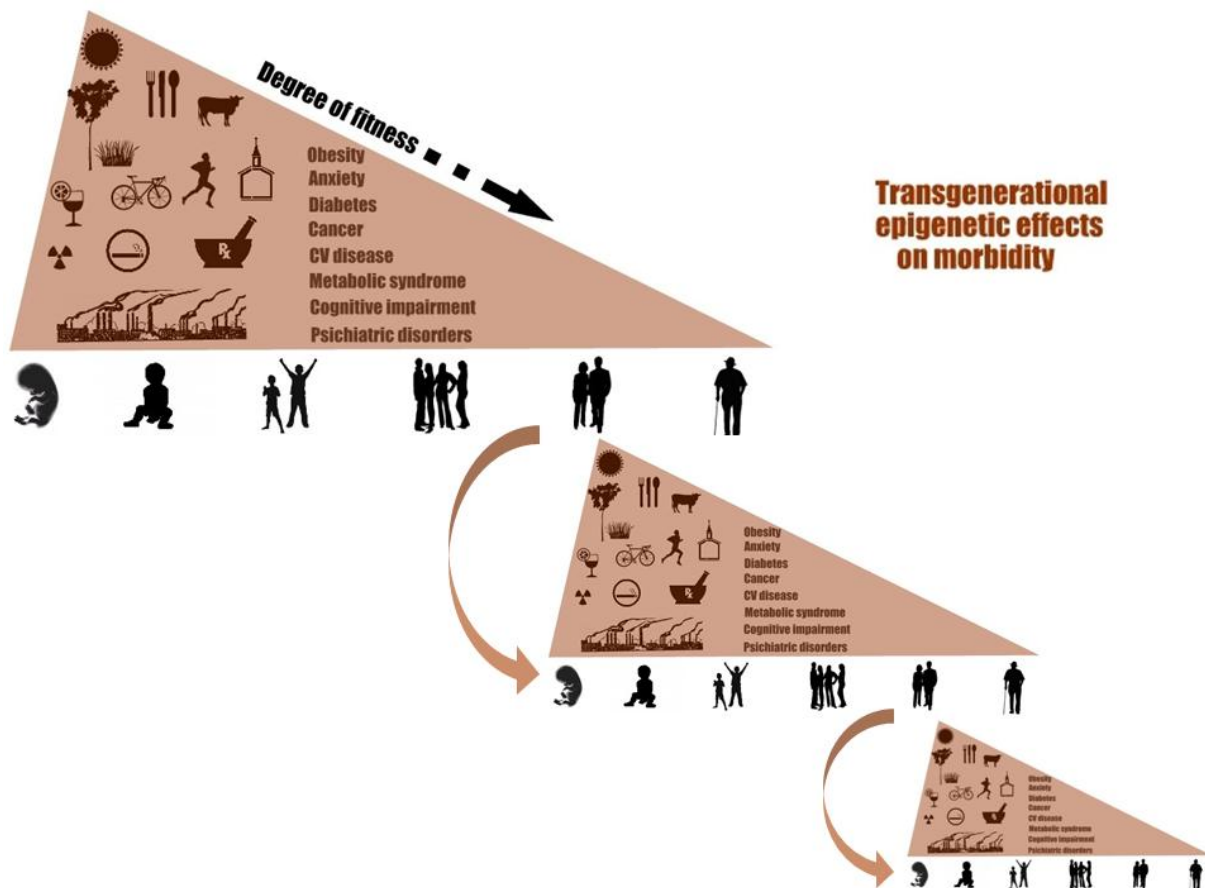
Macro and micronutrient perinatal exposure are some of the most investigated determinants of later life outcome. In the pregnant rat, micronutrient levels like vitamin B12 and folate, two major mediators of brain development, memory and learning, modulated nerve growth factor (NGF) and brain-derived nerve growth (BDNF) in the pups' brains [28]. A postnatal replenishment of these micronutrients did not reverse the decrease of the investigated neuromodulators, but a prenatal omega-3 fatty acids supplementation proved to be protective. The beneficial roles of docosahexaenoic acid (DHA) in establishing a better adapted phenotype was also shown by measuring memory and learning,

oxidative damage, apoptosis, and mitochondrial metabolism in the rat offspring's hippocampus in response to restraint stress [29]. Also, the alpha-linolenic (ALA) rich flaxseed oil maternal supplement correlated with better spatial memory performance and higher DHA levels in the same brain region for the rat pups [30]. The gestational ALA deficiency was associated with lower neurogenesis in the dentate gyrus of C57BL/6J mice pups, even with ALA supplementation during lactation versus a deficient diet [31].

Studies in humans are limited, but there is evidence that DNA methylation levels vary with age in specific

tissues and during certain periods of development and maturation. There is a positive correlation between age and DNA methylation in different brain regions (frontal cortex, temporal cortex, pons, and cerebellum) [32]. This was confirmed by a more recent study examining DNA methylation in ~14,500 genes in postmortem brain tissues collected from human prefrontal cortex. Numata et al found a decreased rate of change and inversion of methylation, from a fast changing process with bidirectional methylation during the fetal period, to a slower pace and mainly increased DNA methylation

during childhood and adulthood [33]. Additionally, other epigenetic factors show a similar pattern, with neuronal chromatin remodeling occurring in early childhood as determined by assessing the trimethylated histone H3 lysine 4 (H3K4) in the human prefrontal cortex [34]. This suggests that brain development is also a vulnerable process from an epigenetic perspective and allows the possibility that environmental exposure with impact upon DNA methylation might have a stronger effect during the childhood period than later in life.



**Figure 3. Transgenerational epigenetic effects on morbidity.** Multiple perinatal factors can have a transgenerational, lifelong impact on morbidity. Environmental triggers such as pollution, drugs, nutrition, smoking, radiation, exercise and social interaction, are hypothesized to have major effects on the adaptive fitness of the individual, and the risk of pathological aging in future generations. This figure depicts how these triggers can impact the risk of disease, in addition to their phenotype defining role. Early intervention on these factors can improve the health outcome later in life not only for the exposed generation, but also for the next. (Infogram created with online tools available at <http://www.easel.ly/>).



## Metabolic consequences

Metabolic effects transmitted between generations are not necessarily initiated by diet alone, but this is the most investigated type of trigger. Interestingly, food selection and appetite can also be altered by in utero experiences. In rats, a high fat/low protein cafeteria diet administered during pregnancy and lactation determined higher preference and intake for a high fat food in the pup, when compared to the control group [35]. The concept that the maternal perinatal diet will influence food choices in the offspring is supported by the observation of modifications in the development of pathways regulating motivation and reward, such as gene expression of the  $\mu$ -opioid receptor and dopamine active transporters and receptors [35, 36]. By erroneously setting these nutritional preferences from the fetal period through maternal metabolic effects, the future individual is already being conditioned to a life of high risk for metabolic syndrome, insulin resistance, and cardiovascular disease. This is not necessarily an irreversible process, as shown by prenatal nutritional intervention. Switching obese dams to a low fat diet during pregnancy prevents the elevated adipose accretion in the pups [37]. However, a different metabolic fate is formed after birth, based on maternal diet, and irrelevant of the present environment, underscoring the importance of an early intervention. A longitudinal study of the hepatic transcriptome in a porcine model exposed sows to low versus adequate protein intake during gestation. It was observed that the first generation of piglets had differential expression of genes involved in the amino acid and lipid metabolism, even with identical postnatal diet [38]. Diet-conditioned maternal programming has additional effects on adjusting metabolic pathways and modulating liver function. Hepatic senescence, as determined by the expression of inhibitor of cyclin dependent kinase 4a, was not reversed by a postnatal control diet after maternal gestational fat intake [39]. This aging effect was associated with low expression of genes involved in the antioxidant defense, such as glutathione peroxidase-1, Cu/Zn superoxide dismutase and paraoxanase enzymes. Gestational high fat in an obesity-resistant mouse model was associated with changes in histone H3 and H4 acetylation and methylation in the promoter or coding region of the gluconeogenic gene phosphoenolpyruvate carboxykinase 1, adipokines (adiponectin and leptin), and higher expression of other relevant genes and transcription factors such as glucose 6 phosphatase and the leptin modulator CCAAT/enhancer-binding protein alpha [40, 41]. This separation of maternal obesity and insulin resistance, from the fetal setting shows how the

perinatal impact of diet alone, and not just a potential detrimental maternal phenotype, can determine changes later in life. However, obesity and overnutrition before mating and during gestation in rats induced glucose intolerance in the adult male, which was reversed by chow feeding [42]. A very recent study observed the quantity and type of macronutrients a rat will consume in the second part of gestation (day 12 to birth), in an attempt to simulate the excessive snacking behavior found in pregnant women during this period. Male offspring, followed up to 20 weeks into adulthood, were hyperglycemic, hyperinsulinemic and had increased gene expression for the hypothalamic Ob-Rb leptin receptor in the carbohydrate supplemented group [43]. These findings, reported versus an ad libitum control, and not reproduced with a high fat supplemented diet, supported the concept of how a certain type of trigger can determine a lasting metabolic change when applied during a sensitive developmental period.

High cholesterol levels in adulthood, with all the adjacent risks, can also be linked to the maternal environment. A low protein diet administered to a rat model during gestation and lactation determined intra-uterine growth restriction with hypercholesterolemia in both male and female offspring initially, which then persisted during late adulthood for males only [44]. The suspected mechanism is through posttranslational histone modifications (H3 decreased acetylation and increased methylation) in the *Cyp7a1*, the main cholesterol to bile acids converter.

In humans, micronutrient supplementation (including vitamins A, B6, B12, C, D, folic acid, and zinc) in a Gambian population determined sex-specific methylation changes in imprinted genes such as *IGF2R* (insulin like growth factor 2 receptor) and *GTL2-2* (gene trap locus 2) at birth, as detected in cord blood, but without subsequent confirmation at 9 months of age [45]. Differences were detected in both treatment and placebo groups in autosomal genes involved in lipid, glucose metabolism, and cardiac function [46, 47]. We posit that studies such as this one will start clarify the extraordinary complexity of changes that are initiated early in development, and that can either persist throughout life, or disappear soon after birth.

Adding to the effects observed in behavioral studies discussed earlier, the apparently omnipresent BPA also has implications in the metabolic fate of those exposed to it. Environmental exposure of the gestating rat to BPA, in concentrations similar to those in humans, determined insulin resistance with altered glucose tolerance, and higher levels of insulin and glycerol in male offspring in late adulthood [48]. This xenobiotic acts as a demethylating agent in genes that control the maturation

of different systems, including imprinted genes, with its effect being reversed by a diet rich in methyl groups or supplementation with genistein [49, 50].

We should also emphasize the importance of the paternal inheritance [51], which is less investigated than the transgenerational changes in the maternal line. A study performed on F0 generation boars fed with a high-methylating micronutrient diet found significant differences versus controls in the F2 generation for gene expression in muscle, liver and kidney, with an impact on lipid metabolism and other metabolic pathways, as well as quantitative DNA methylation differences in some, but not all, inquired promoters [52]. Dunn et al showed that maternal high fat diet during pregnancy and lactation determines increased body length and impaired insulin sensitivity in F1 and F2 of mice generations transmitted through both maternal and paternal lineage, but subsequent transmission to F3 of the higher body size and weight phenotype was restricted only to females, and being transmitted just through paternal lineage [53].

#### **Other epigenetic effects with implications in pathological aging**

We have to consider the perinatal vulnerability of the developing organism to different epigenetic triggers, and the multiple effects of a single compound upon different organ systems. The stability of epigenetic players such as H3K4 methyltransferases is already established in regulating expression of genes involved not only in neuronal fate, but also in defining function in cells such as cardiomyocytes [54], with a significant impact on cardiac. The effect of a gestational diet high in fat or fructose, or low in protein, regardless of the postnatal diet, can impact more than upon cognitive and metabolic systems. Cardiovascular and renal involvements are very important determinants of later life health, and recent studies indicated how such outcomes are influenced by maternal environment [55]. The fat excess in the maternal diet, and the type of consumed fats, can have implications in the regulation of vascular tone in the offspring through fatty acids desaturases 1 and 2 alterations in the arterial wall [56]. A sawflower oil, high fat maternal diet rich in omega-6 polyunsaturated fatty acids during C57Bl/6 mouse gestation, followed by chow for 8 weeks after birth, was correlated with lower levels of cardiac arachidonic acid (AA) and linoleic acid (LA) in the offspring [57]. These non-reversible changes for the duration of the study impacted the cardiac function later in life, with LA shown to be relevant in establishing cardiovascular risk factors [58].

Cytochrome P450 3a, an important enzyme in drug metabolism, was decreased in mice exposed to high fat *in utero*, starting immediately after birth and continuing for at least 12 weeks, with the subsequently administered control diet not reversing this trend. This finding, if replicated in humans, could have important implications for the future patient, connecting gestational environment with drug sensitivity, and with the potential of individualized pharmacological care for a segment of vulnerable subjects [59]. Exposure to BPA promoted prostate cancer later in life in a rat model, through neonatal exposure associated with stable epigenetic alterations during adult life, which involved the DNA hypomethylation of the nucleosome binding protein-1 (Nsbp1) promoter, and differential expression of Dnmt3a, Dnmt3b, and Mbd2 [60]. These three very important genes that participate to the control of the epigenetic machinery, were up-regulated by maternal low protein diet in the same animal model, with hypermethylation of the imprinting control region (ICR) in the Igf2/H19 locus and high Igf2 expression, but partially reversed by folate supplementation [61]. Another important affected function is reproduction, with changes leading to premature infertility. Paternal high fat diet in a F0 C57Bl/6 mice generation determined obesity and decreased reproductive health in the F1 offspring, and perturbed sperm function, reduced its motility, and associated with higher levels of reactive oxygen species in F2 [62]. These changes were transmitted through both lines, with more drastic effects in males.

#### **Conclusion**

Perinatal epigenetic alterations should be considered as potential biological markers for determining the type of exposure, and research is still needed to determine the potential diagnostic value of epigenetic profiles as predictors for disease. Research focused on differential DNA methylation regions (DMRs) identified specific changes attributed to particular chemicals found in our environment [63]. The reviewed literature indicated a transmittable epigenetic component in all these types of disease. Not all studies concur in regard to the concept of stable transmitted patterns, with some showing contradictory results and variable changes that lack consistency between generations, or which cannot explain the observed phenotype [64]. The environment will still send signals throughout the lifespan but some adaptive patterns will probably not be reversed. The aim of investigating the long-lasting, multigenerational, effects of environmental exposure on humans is very ambitious and extremely difficult to accomplish. For

now, animal models and limited human studies have proven the existence of this phenomenon, and its resistance to change, or in some cases, reversibility. The question is, in our fast changing habitat, how can one take into account numerous concomitant signals that will have synergistic or antagonistic effects, and how will it conclude the presence of a beneficial adaptive trend or a dysfunctional status quo. Considering the complexity of interpreting a multitude of stimuli during vulnerable phases of human development, it is conceivable that the only relevant studies in this area of research will be supported by adequate biostatistical algorithms that can interpret thousands of variations in a dynamic environment. In addition, studies that follow-up multiple generations are essential in order to demonstrate or reject the hypothesized epigenetic inheritance and its roles upon the health status of next generations. However, the existing evidence has solidly established that parental environments can alter the phenotypes of next generations, whether as a consequence of epigenetic inheritance, or directly acting via metabolic imprinting.

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